

CAR-M are professional antigen presenting cells, we developed an immunocompetent animal model to evaluate the potential for induction of a systemic anti-tumor immune response.

**Methods** Murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adenoviral vector Ad5f35. CAR-M were phenotypically and functionally evaluated in vitro and in syngeneic models. To evaluate CAR-M efficacy in an immunocompetent animal model, BALB/c mice were engrafted with CT26-HER2+ tumors (single-tumor model) and were treated with intratumoral CAR-HER2 or untransduced (UTD) macrophages. To evaluate epitope spreading, we simultaneously engrafted BALB/c mice with CT26-HER2+ and CT26-Wt tumors on opposite flanks (dual-tumor model), and treated mice with CAR-M or controls into the CT26-HER2+ tumor only. Peripheral and tumor-infiltrating immune cells were phenotypically and functionally characterized.

**Results** In addition to efficient gene delivery, Ad5f35 transduction promoted a pro-inflammatory (M1) phenotype in murine macrophages. CAR-M, but not control UTD macrophages, phagocytosed HER2+ target cancer cells. Anti-HER2 CAR-M eradicated HER2+ murine CT26 colorectal and human AU-565 breast cancer cells in a dose-dependent manner. CAR-M increased MHC-I and MHC-II expression on tumor cells and promoted tumor-associated antigen presentation and T cell activation. In vivo, CAR-M treatment led to tumor regression and improved overall survival in the CT26-HER2+ single-tumor model. In the dual-tumor model, CAR-M treatment cleared 75% of CT26-HER2+ tumors and inhibited the growth rate of contralateral CT26-WT tumors, demonstrating an abscopal effect. CAR-M treatment led to increased infiltration of intratumoral CD4+ and CD8+ T, NK, and dendritic cells – as well as an increase in T cell responsiveness to the CT26 MHC-I antigen gp70, indicating enhanced epitope spreading. Given the impact CAR-M had on endogenous T-cell immunity, we evaluated the combination of CAR-M and anti-PD1 in the CT26-HER2 model and found that the combination further enhanced tumor control and overall survival.

**Conclusions** These results demonstrate that CAR-M therapy induces epitope spreading via activation of endogenous T cells, orchestrating a systemic immune response against solid tumors. Moreover, our findings provide rationale for the combination of CAR-M with immune checkpoint inhibitors. The anti-HER2 CAR-M CT-0508 will be evaluated in an upcoming Phase I clinical trial.

#### REFERENCE

1. Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 2020;**38**(8):947–953.

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#### DEVELOPMENT OF NOVEL CELLULAR THERAPEUTICS FOR METASTATIC AND PRIMARY CNS MALIGNANCIES

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**Background** Treatment of solid tumors with cell therapeutics will require optimal T cell persistence, fitness, and trafficking. Heterogeneous solid tumors will also have to be attacked through multiple antigens simultaneously in order to prevent resistance linked to loss of antigen expression. Here we use chimeric antigen receptor (CAR) T cells that secrete bridging

proteins that act as CAR-T engagers to create an optimal platform for attacking solid tumors in the CNS.

**Methods** Lentiviral vectors encoding an anti-CD19 CAR and secreted bridging proteins were created. The bridging proteins contained the CD19 extracellular domain, which is the target for the CAR, and anti-tumor antigen binding domains derived from antibodies (scFv and llama VH). The resulting anti-CD19 CAR T cells secrete the bridging proteins. These candidate cell therapeutics were evaluated for antigen binding and induction of antigen-specific cytotoxicity. An anti-CD19 CAR that secretes a CD19-anti-Her2 bridging protein has moved into development. Using the CD19-anti-Her2 bridging protein as a core module, we have begun evaluating a series of multi-antigen bridging proteins.

**Results** CAR-CD19 T cells that secrete bridging proteins have potent cytotoxic activity against single- and multi-antigen-positive cells. ALETA-002 is the lead candidate lentiviral vector construct encoding the anti-CD19 CAR domain and the CD19-anti-Her2 bridging protein, and has entered a GMP viral particle development campaign. This therapeutic will be systemically administered to Her2-positive breast cancer patients who are relapsing with CNS metastases. Next, multi-antigen bridging proteins encoding an anti-Her2 scFv and anti-B7H3, anti-B7H6 or anti-IL13Ra2 llama VH were assayed for potency. Lead candidates for development for the treatment of primary CNS malignancies were identified and are being manufactured at pilot-scale in 4-plasmid lentivirus production runs.

**Conclusions** The use of anti-CD19 CAR T cells that can expand off of the normal CD19-positive B cell pool enables tumor-antigen independent persistence, fitness and robust trafficking into the CNS. The use of small, modular bridging proteins allows us to leverage anti-CD19 CAR T cells and use these to attack solid tumor antigens that are present on CNS resident cancers and on CNS metastatic lesions. Novel cell therapeutics for the treatment of Her2-positive CNS metastases and heterogeneous primary CNS malignancies including glioblastoma and the pediatric gliomas have been developed.

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#### TUMOR-RESPONSIVE, MULTI-FUNCTIONAL GENETICALLY-ENGINEERED NATURAL KILLER CELLS FOR IMMUNOTHERAPY OF GLIOBLASTOMA

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**Background** Despite aggressive treatments and care, the median survival for GBM patients is 14.6 months, which has only modestly improved over the past several decades, highlighting the need for new therapeutic approaches. NK cells, innate cytotoxic effectors, are showing potential for cancer immunotherapy for GBM.<sup>1-3</sup> However, tumor antigen heterogeneity and a severely immunosuppressive tumor microenvironment (TME) have rendered GBM highly resistant to most single antigen-based NK monotherapies.<sup>4-6</sup>

**Methods** To overcome these challenges, our solution has been to develop a first multifunctional immunotherapy for GBM based on genetically-engineered NK cells bearing multiple simultaneous anti-tumor functions, including local tumor responsiveness and the ability to avoid antigen escape. The activity of these lentivirally-transduced multi-functional NK (E-